Final Synopsis
(2017-2024)

Enzymatic Intervention for The Production of Medium-Chain Fatty Acids Enriched Product to Control Metabolic Disorders

Submitted to

Department of Biochemistry
Mangalore University

FOR THE AWARD OF THE DEGREE OF
DOCTOR OF PHILOSOPHY IN
BIOCHEMISTRY

By

Moorthy Karthika Selvi
No.MU/EXB/Ph.D./CR.175/ Biochem./2018-19/E.13

Under the supervision of

Dr. P. Vijayaraj
Principal Scientist, Department of Biochemistry
CSIR-Central Food Technological Research Institute
Mysore- 570020

Name of the Candidate	Moorthy Karthika Selvi
Title of Thesis	Enzymatic intervention for the production of medium-chain fattyacids enriched product to control metabolic disorders
Name of Research Supervisor	Dr.P.Vijayaraj
Name of the Institution	Department of Biochemistry, CSIR- Central Food Technological Research Institute
Date of Registration	No.MU/EXB/Ph.D./CR.175/ Biochem./2018-19/E.13 01-06-2017
Date of Colloquium	01-04-2024

Enzymatic intervention for the production of medium-chain fatty acids enriched product to control metabolic disorders

Introduction:

Lipids play a crucial role in our diet, serving as essential components. Lipids contribute to desirable organoleptic, nutritional, physical, and biological. Dietary lipids, particularly triacylglycerols (TGs), are comprised of a glycerol backbone esterified with three fatty acid (FA) chains. Based on their structural characteristics, these fatty acids can be categorized into saturated fatty acids (SFAs), monounsaturated fatty acids (MUFAs), and polyunsaturated fatty acids (PUFAs). Triglycerides, also known as triacylglycerols, are referred to as oils when in a liquid state (MUFA/PUFA) and as fats when in a solid state (SFA) at room or ambient temperature (Dorni et al., 2018). According to the Indian Council of Medical Research (ICMR), the Recommended Dietary Allowance (RDA) for fat intake for Indian adults is set at 30 grams per day.Recommended dietary allowances (RDA) for fat and fatty acid intakes are based on global standards aimed at preventing lifestyle diseases. The fat composition of a diet varies according to ethnic and regional factors as well as economic status. The common dietary lipids include triglycerides, glycolipids, phospholipids, sterols, and waxes, found in various sources such as vegetable (seed) oils, meat products, dairy products, cereals, fatty fish, and fish oils (Rustan and Drevon, 2001). Fats and oils, the major dietary lipids (Akoh & Min, 2002), constitute 30 to 40% of total energy intake (Willett, 1998). The primary sources of dietary lipids are fats and oils derived from oil seeds (such as sunflower, sesame, safflower, mustard, and flaxseed), fruits (like palm, avocado, and olive), beans (such as soybean), nuts/kernels (including groundnut, coconut, palm kernel, cashew, almond, and walnut), and cereals (such as rice, wheat, and corn). Naturally occurring vegetable oils predominantly consist of triglycerides (TAG) as a major constituent, with minor amounts of diacylglycerol (DAG) and

monoacylglycerol (MAG). Oils provide 9 kcal/g more energy than proteins and carbohydrates (Flickinger and Huth, 2004). A significant portion of human diets comprises vegetable oils (Zambiazi et al., 2007), functioning as transporters for fat-soluble vitamins (A, D, E, and K) and essential fatty acids (EPA, DHA, linoleic, and oleic acids), vital for growth. Fats and oils play crucial roles in maintaining a balanced diet, but unused fat can accumulate as storage fat, leading to obesity and related complications. Consumption of an imbalanced ratio of saturated, monounsaturated, and polyunsaturated fatty acids with in fat can contribute to chronic diseases. India imports more than 60% of its vegetable oils. However, it ranks third worldwide in the number of obese individuals and occupies first place in diabetes cases, largely due to its present unhealthy eating habits and sedentary lifestyle. By 2050, It is expected that over 70% of the world's population will reside in urban areas,representing a significant increase from the current proportion of over 50% (WHO, 2014). The quality of fats and oils not only influences overall product quality but also plays a pivotal role in determining nutritional aspects, including health benefits. The health advantages of dietary lipids are primarily associated with the specific types of fatty acids contained within them. Nutritional and health benefits of dietary fats can be achieved through lipid modification. The process of lipid modification involves the rearrangement or esterification of specific fatty acids at particular positions on the glycerol backbone during the synthesis of triacylglycerols. This modification helps achieve the desired lipid composition to meet specific functional requirements in both food and nutrition.

Triglycerides can be categorized based on the carbon atom count into short-chain triglyceride (SCT), medium-chain triglyceride (MCT), or long-chain triglyceride (LCT) species. The type of fatty acids that form an aliphatic tail and have varying numbers of carbon atoms and saturation determines the type of triglyceride. Long-chain triglycerides represent the most common dietary fat, with each aliphatic tail containing 12–22 carbon atoms. Short-chain triglycerides have aliphatic tails with fewer than 6 carbon atoms. Medium-chain fatty acids

(MCFAs), such as lauric acid (C12:0), comprise fatty acids containing between 6 and 12 carbon atoms and are classified as a subgroup of dietary triglycerides (Turpeinen, A., &Merimaa, P. 2011). Lauric acid cannot be categorized as a Medium Chain Fatty Acid (MCFA) due to its displaying intermediate characteristics, falling between those of MCFAs and LCFAs. This is attributed to its absorption rate of 70-75% and transportation by chylomicrons (Denke, M. A., & Grundy, S. M. 1992; Wallace, T. C. 2019). MCFA and LCFA provide around 8.3 and 9.2 kcal/g (Krishna, A. G., et al., 2010, Pandya and Haenlein 2009; Esperon Rojas *et al*., 2022). Medium-chain triglycerides (MCT) are formed when MCFA binds to triacylglycerol. MCFAs, Fatty acids with carbon atom counts ranging from 6 to 12, known as medium-chain fatty acids (MCFAs), are found in various dietary sources like coconut oil, palm kernel oil, bovine milk, babassu oil, cohune oil, and cuphea oil. These sources are notable for their significant concentrations of medium-chain fatty acids (C8:0–C12:0) (Babayan, 1987; Petrauskaite, Greyt, and Kellens, 2000; Jensen, 2002, Ashton *et al*., 2021; Duttaroy 2021; Mett and Muller 2021, Zentek *et al*., 2011). Other sources of LCFAs-rich oils include rice bran, sunflower, safflower, sesame, mustard, palm oil, and soybean. These dietary fats, whether used in industry or at home, include LCFAs. Excessive consumption of these dietary fats is linked to several chronic illnesses, including obesity, cancer, cardiovascular disease, and other health problems primarily observed in developed countries (Liu et al., 2020; Jin et al. 2023; Li et al. 2023).

MCFAs gaining attention for their nutritional therapy and functional benefits. Synthetically MCFA and glycerol undergo an esterification process to produce MCT (Jadhav and Annapure 2021a; Jadhav *et.al*., 2021b). Since 1994, MCTs have been designated Generally Recognized as Safe (GRAS) for food consumption by the USFDA (Traul et al., 2000). Renowned for a plethora of health advantages, MCTs have emerged as a prominent 'nutraceutical fat' (St-Onge and Jones, 2002). Initially introduced as a potent energy source for various clinical nutrition

applications, including parenteral feeding, obesity management, and fat malabsorption, MCTs are also integrated into infant food formulations (Lee, 1986; Yuan et al., 2020). Due to structural disparities, MCTs and long-chain triglycerides (LCTs) exhibit distinct absorption and transportation mechanisms (Marten et al., 2006). MCFAs are rapidly metabolized into ketone bodies, providing an immediate energy source to the body (Bach & Babayan, 1982; Marten et al., 2006). Notably, MCTs are reputed to lower blood cholesterol levels and facilitate weight loss. Presently, there are limited comparative analyses of edible oils to evaluate MCFA levels. Commonly used edible oils include coconut oil (CNO), virgin coconut oil (VCO), refined palm kernel oil (RPKO), palm oil (PO), rice bran oil (RBO), and sunflower oil (SFO). However, MCT oil is relatively expensive compared to other oils, necessitating the development of cost-effective formulations through enzymatic methods and interesterification processes. Structural lipid modifications can enhance the MCFA content in commercial oils. Research has explored the commercial production of MCFAs, particularly in coconut oil (CNO) and refined palm kernel oil (RPKO) (Petrauskaite, Greyt, and Kellens, 2000). These modified oils (MCFAs) serve as a foundation for various products, including bakery and confectionery items. However, in the present study, we proposed oil-in-water emulsified products i.e., mayonnaise, even it can be used as a table spread. The combination structured lipid (fat content less than 50%) with the prebiotic is not available for the human consumption. The supplementation of the product to the normal mice will reveal the maintenance of healthy body weight whereas the diet-induced obese mice will show the positive impact on circulatory lipids. The safety and toxicological parameters to be studied along with the lipid metabolic profiling. MCFAs enriched could provide a novel source for creating energy-rich food formulations.

The proposed study addressed the globally alarming issue of obesity and its complications. It resulted from a prolonged imbalance between energy intake and energy

expenditure. The pharmaceutical industry had invested considerable efforts in developing anti-obesity drugs, with only pancreatic lipase inhibitors approved and authorized for obesity treatment (Haslam D, 2016). Dietary control had emerged as a safer option for both weight maintenance and reduction. However, the replacement of fats with carbohydrates had been associated with the onset of diabetes and other chronic diseases (DiNicolantonio, 2014). Consequently, the objective of the study was to develop healthy vegetable oils and related products to substitute weight-gaining fats and oils. The main objective of the present study is explained in Subsequent chapters.

Objectives of the present study:

- Enzymatic modification of the vegetable oil for the preparation healthy emulsified products such as oil-in-water (O/W).
- Physiochemical characterization and optimization of product formulations with natural molecules to control the circulatory free fatty acids.
- *In vivo* validation of the efficacy as well as safety evaluation in both normal and obesity-induced insulin resistant animal model.

CHAPTER 1: General Introduction

This chapter offers a concise introduction to the importance of medium-chain fatty acids and their applications from various sources. Additionally, it elucidates the definition of the problem and the purpose of the study.

CHAPTER 2: Review of Literature

The second chapter reviews the origin of Medium Chain Triglyceride oil and its different sources of Medium Chain Fatty Acids (MCFAs) content. It briefly describes the importance of structured lipid through blending and enzymatic inter-esterification. The chapter covers the preparation of healthy oil-in-water emulsified products, such as low-fat prebiotic mayonnaise, designed to maintain a healthy body weight and control circulatory free fatty acids. Additionally, a brief overview explains the biosynthesis pathway for both medium chain triglyceride and long chain triglyceride oils. The chapter further provides an overview of the in vivo evaluation and biochemical approach to MCFAs-based products.

CHAPTER 3: Enzymatic modification of the vegetable oil for the preparation healthy emulsified products such as oil-in-water (O/W).

This chapters covers the first objective of the thesis work in which screening selection of medium chain fatty acids containing vegetable oils was performed and based on the reported evidences we have short listed five major plant sources. Coconut oil, Palm Kernel oil Babassu oil, Cohune oil, Tacum oil and Cuphea contains significant amount of short and medium chain fatty acids. Cuphea is often used as crop rotational plant mainly for wheat and corn to improve soil quality and yield. However, due to the low availability of Babassu oil, Cohune oil, Tacum oil, Cuphea only Coconut oil, and Palm Kernel oils were selected for the further study. Along with these oils we have studied about the other commercial oils such as Coconut testa oil, virgin coconut oil, rice bran oil, sunflower oil and palm oil. The fatty acid composition of MCFA content in Coconut oil 78.83%, Virgin Coconut oil 66.65%, Refined Palm Kernel Oil 58.25% and Coconut testa oil 41.96%. Among know different edible oils Coconut oil is one which is rich in MCFAs, it has antidiabetic property by balancing blood sugar levels. (Marten *et.al*., 2006) and health benefits have been attributed to it. Moreover, CNO is becoming more and more recognized as a possible cancer controlling and chemotherapy-protective agent (Boemeke, L., *et.al*., 2015). Coconut testa oil is often by product rich in plant phenolics, antioxidants. Coconut testa oil was found to have more unsaturated fatty acids in comparison with different fractions of Coconut oil (i.e. CNO and VCO). In Coconut testa oil Lauric Acid (C12:0) was 33.24%, whereas Caprylic Acid (C8:0) and Capric Acid (C10:0) were 4.81% and 3.55%, respectively.

Oxidative stability of five edible oils: Coconut Oil (CNO), Palm Oil (PO), Rice Bran Oil (RBO), Sunflower Oil (SFO), and Fish Oil (FO) at a constant temperature of 90°C was determined. The observed results revealed distinct oxidative stability profiles for each oil. CNO exhibited the highest stability with an Oxidation Induction Time (OIT) of 136 hours and 38 minutes, highlighting its suitability for high-temperature cooking. PO and RBO showed moderate stability (OIT: 49h 11m and 26h 15m). SFO displayed lower stability (OIT: 10h 9m), while FO was the least stable (OIT: 54m) due to its high unsaturated fatty acid content. Collectively, the OXITEST provides valuable insights into the oxidative stability of fats and oils, standing out for its real-time, accelerated testing capabilities, and providing faster insights into oxidation behaviour compared to traditional methods.

Enzymatic production of MCT

Coconut and palm kernel were chosen as a source of medium chain triglycerides and blending of these oils was done, based on the MCFAs content. These oils were blended CNO: RPKO ratio and further used for inter-esterification. These blended oils were further esterified by using commercial enzymes (Enzy-I). The blended oil esterification with glycerol (CNO: RPKO WG) and without glycerol (CNO: RPKO WOG) was done. Also in the study "CNO +Enzy1" was esterified. In "CNO: RPKO" blend oil 92% TAG content was initially observed. After esterification "CNO: RPKO WOG", "CNO +Enzy1" showed 88% TAG content. whereas "CNO: RPKO WG" showed 67% TAG content respectively. "CNO: RPKO WOG" showed highest amount of lauric acid 53%. After esterification triglycerides were separated from TLC and its fatty acid profiling was observed. C6:0, C8:0 medium chain fatty acid were not observed in the enzyme treated oil Whereas it's observed in initial oils. From the above experiments, we concluded that TAG rich Medium chain triglyceride was not obtained.

Further, in our study we have selected fatty acids of C8:0 and C10:0 obtained from coconut oil (CNO) source. These fatty acids from CNO source was interesterified. The reaction was conducted using different commercial enzymes (Enzy-I, II and III) at different time intervals. Even after enzyme treatment complete free fatty acids conversion to TAG was not observed. So, further standization of experiment has been done. Fatty acid profile C8:0 (45%), C10:0 (54%) was found be similar in all three enzymes. Whereas "Enzy-III and Enzy-I" has showed better results, complete conversion of fatty acid to triacylglycerol has been done at 6hr. In "Enzy-II" no reaction was observed fatty acids remained same. As per physical appearance and odour the enzyme "Enzy-I" was selected for the further study. Purification of triacylglycerol (TAG) was performed by solvent extraction method, by repeated solvent wash. In the 4^{th} wash there is no MAG present, DAG is slightly present (20%), TAG (80%) respectively. The purified TAG (80%) rich Medium Chain Triglyceride (MCT) for which fatty acid profiling consists of C8:0 (49.9%) C10:0 (50.0%) respectively.

CHAPTER 4: Physiochemical characterization and optimization of product formulations with natural molecules to control the circulatory free fatty acids.

Structured Lipids are prepared by blending and inter-esterification methods. The oil blends were prepared by using purified Medium chain triglyceride (MCT) and vegetable oils at different ratios. From the fatty acid profile, we have concluded that the blending of CNO: MCT showed 75% Medium chain fatty acids (MCFA'S), RBO: MCT 63% MCFA'S, CNO: MCT

66% MCFA'S, MCT: RBO 90% MCFA'S, MCT: RBO 59% MCFA'S, whereas MCT: RBO showed highest 90% MCFA'S when compared to other blends. Considering the cost effectiveness, we have taken "CNO: MCT" (BC), "RBO: MCT" (BR) blends for further enzymatic inter-esterification. From the prepared blends enzymatic Inter-esterification was carried out by using enzyme (Enzy-I), in which the fatty acids for inter-esterified "CNO: MCT" 62% MCFA'S in which it showed 35% Lauric (C12:0), "CNO: MCT" IC 70%, MCFA'S "RBO: MCT" (IR) MCFA'S 44%. Inter-esterification is carried out to see any changes in TAG species. "MCT" oils showed that no DAG formation in "BC" samples. DAG and FFA'S formation is observed in "IC". When rice bran oil blended with MCT "IR" showed more DAG. From the above experiments concluded that MCFA'S was shown more in 'BC", "IC", whereas "BR", "IR" was chosen for non-coconut oil consumers.

The physicochemical characteristics analysis of individual oils i.e., MCT, CNO, RPKO, RBO, BC, IC, BR and IR. Was done. Free fatty acid content is higher in interesterified oil "IC" (3.0%) and "IR" (3.76%). Peroxide value is slightly higher in "IC" (1.98), "BR" (1.97), and "IR" (1.98). Saponification value of "MCT" was 326.6 mg KOH/g while that of "CNO" and "RBO" 263.11 & 186.33 mg KOH/g respectively. Unsaponifiable matter (USM) is higher in "RBO" (3.78%). Iodine value for "RBO" was high (97.46 cg I_2/ g) due to the presence of unsaturated fatty acids. "MCT" showed a lesser amount of iodine value (0.11 cg I_2/ g) due to the presence of saturated fatty acids. Generally, vegetable oil contains TAG as the major portion of glycerides, "MCT" (100%), "CNO" (94.41%), "RPKO" (91.34%) "RBO" (88.58%), "BC" (94.11%), and "BR" (91.11%). DAG content was found to be higher in "RBO" (10.05%) and "BR" (6.01%). In intersesterified oils TAG was found to be lesser, whereas DAG content was higher, and a slight increase in MAG was observed when compared to initial and blended oils. Phytonutrient content was analyzed to check the phytosterols, tocopherol and tocotrienol content. MCT oil doesn't contain any phytonutrients upon blending and interesterification. MCT rich oils showed nutraceutical potential value. TAG profile for initial, blended, and interesterified oils were estimated by HPLC and HRMS. MCT oil showed only four major peaks Tricaprylin (C8:0), Tricaprin (C10:0), Dicaprylin monocaprin (C8:0-10:0-8:0) and Dicaprin monocaprylic (C10:0-8:0 -10:0). Dicaprylin monocaprin is the predominant TAG consists of ECN-26 (45.87%). Trilaurin is major TAG which contributes ECN-36 LaLaLa (22.14%) and (20.44%) to be highest in "CNO" and "RPKO". "RBO" contains ECN-46 POL (17.85%) followed by ECN-48 POO (14.56%). "CNO' showed very lower quantity of Tricaprylin (C8:0), Tricaprin (C10:0), Dicaprylin monocaprin (C8:0-10:0-8:0) and Dicaprin

monocaprylic (C10:0-8:0 -10:0). whereas in “RBO”, MCTs are not present. Upon blending and interesterification (CNO and RBO) showed the increase in MCFAs. Dicaprylin monocaprin (C8:0-10:0-8:0) showed highest in BC (20.5%), whereas in IC Tricaprin (C10:0) was highest 17.49% upon interesterification we could observe elongation of long chain fatty acids. Both HRMS and HPLC showed atmost similar observations.

Production of mayonnaise enriched with natural molecule to control circulatory free fatty acid.

Mayonnaise is a semisolid oil-in-water contains egg yolk or whole egg to emulsify the oil to stabilize the continuous aqueous phase and a dispersed oil phase. Many commercial mayonnaises are made with high oil (60 -70 %) concentrations, which raise the risk of several various diseases because of the connection to health problems. In the present study to evaluate the effect of medium-chain triglycerides (MCTs) blended with edible oils, four types of vegan based mayonnaise was prepared by using 100% “Soya bean oil” (control), 100% “Sunflower Oil” (SFO), BC and BR . Physicochemical, particle size distribution, rheological properties, texture colour, and sensory properties of low-fat prebiotic mayonnaise. Texture analysis of control samples showed highest firmness (3.27) lowest firmness value was observed in MCT enriched formulation and “SFO”. Colour L* a* b* values was observed in different mayonnaise samples control mayonnaise has more L* value (brightness). Similar “SFO” and “BC” showed similar L* values, whereas BR contain Low L* value and more b* value (yellowness). Due to RBO oil content BC showed b* value similar to BR. Protein content (5g) is same in all samples. Fat content varies from (37-41%). Dietary fibre content in control sample (0.34), SFO, BC, BR contains (3.3). Carbohydrate content is high in “BC” (22.98g). The droplet size of all the samples falls in range of 7-10 µm. Samples with smaller droplet size exhibits higher emulsifying properties. The control sample had the smallest droplet size followed by BC, SFO and BR. BC has better emulsifying property equivalent to control. The sensory evaluation demonstrated that the MCFA-containing mayonnaise was acceptable. MCTs are a good substitute to produce the proper physicochemical properties of mayonnaise. The proposed low-fat prebiotic mayonnaise preparation has no synthetic chemical components or harsh chemical processing. However, we claim that dietary management for healthy body weight will help reduce the serum circulatory free fatty acid content.

CHAPTER 5: *In vivo* validation of the efficacy as well as safety evaluation in both normal and obesity-induced insulin resistant animal model

Impact on weight reduction on obesity-induced insulin resistant animal model

In vivo evaluation of the product was assessed in normal C57BL/6J mice. The experiments were initiated with 4-week-old C57BL/6J male mice, which were housed in an air-conditioned animal room with a 12-hour light/12-hour dark cycle in an animal house facility maintained at 25 ± 2 °C and 40–60% relative humidity. The supplementation of the product to normal mice reveals the maintenance of a healthy body and the safety of the product. Mice were randomly divided into five groups, namely Control (regular diet), and five test groups of mayonnaise made up of sunflower oil, coconut oil-based, rice bran oil-based, and commercial mayonnaise (low fat and high fat). The weight of the animals increased in the commercial high-fat mayonnaise groups compared to the control and other treatment groups. The treatment group, particularly CFTRI-formulated mayonnaise-fed animals, showed a similar body weight compared to the control group, indicating that the consumed fat was utilized for energy production rather than storage. The levels of circulatory lipids such as triglycerides, cholesterol, LDL, and VLDL were significantly lower with the "CFTRI formulation" than with commercial and sunflower oil-based mayonnaise. Cholesterol content was higher in SFO-based mayonnaise when compared to the treatment group mayonnaise and was correlated with the control group. As expected, triglyceride levels were high in the HFD group, whereas treatment groups showed lower triglyceride levels. The study demonstrates that CFTRI-formulated mayonnaise in mice maintains body weight and exhibits favourable effects on circulatory lipids compared to commercial and sunflower oil-based counterparts. These findings suggest the potential health benefits of the formulated mayonnaise in promoting weight management and lipid profile improvement.

Impact on weight gain in obesity-induced insulin resistant animal model

C57BL/6J mice (male, 4-week-old) will be randomly divided into two groups: a normal or control diet and a high-fat diet (HFD). To induce obesity, the mice will be fed the HFD, consisting of 60% fat, for 12 weeks. After 12 weeks of dietary manipulation, the HFD group will be further divided into six groups: one with HFD treatment and the remaining five comprising mayonnaise test groups made up of sunflower oil, coconut oil-based, rice bran oil-based, and commercial mayonnaise (low fat and high fat). Obesity-induced animals were treated with formulated mayonnaise, and similar results were observed. Interestingly, a

significant weight reduction was noted with the "CFTRI formulated" low-fat prebiotic mayonnaise. Cholesterol content is high in the HFD group animals compared to other groups; however, the rice bran oil-based group showed very low levels. This clearly indicates that the "CFTRI formulated" low-fat prebiotic mayonnaise has a promising impact on lipid metabolic parameters, and it can be explored for commercial production.

CHAPTER 6: Summary and Conclusion

Urban living has led to unhealthy dietary habits, with over 50% of the population in cities expected to surpass 70% by 2050. Urban environments encourage high-calorie, fatty, and sugary fast-food consumption, increasing obesity rates and related health problems, causing about 2.8 million deaths annually. Sedentary lifestyles are the fourth leading global cause of death. Healthier food alternatives can reduce complications. Fats and oils significantly influence health, linked to their fatty acid composition. Modifying vegetable oils to produce fats with desired fatty acid composition is crucial. Medium-chain fatty acids (MCFAs) offer rapid energy release and health benefits. Medium-chain triglyceride (MCT) oil, rich in MCFAs like caprylic acid (C8:0) and capric acid (C10:0), promotes weight loss, enhances energy, and improves cognitive function. MCFA are absorbed faster than longer-chain fatty acids (LCFA). MCFAs are partly independent of the carnitine transport mechanism into the mitochondria of the liver and are rapidly and exclusively oxidized for energy production. The hydrolyzed products of MCT are absorbed as fast as glucose. Despite the commercial potential, our country lacks MCT production technology, which limits availability and market growth. The present study aimed to develop a technology for MCT oil and blended oils for cooking and provide healthier alternatives to high-fat foods like mayonnaise. We hypothesize that altering fatty acids, glyceride composition, dietary molecules, and nutraceuticals can mitigate weight gain and obesity-related complications. We developed an enzymatic process to produce MCT, MCFA-rich edible oil, and healthy emulsified oil-in-water (O/W) products like mayonnaise.

We identified commercially viable sources of MCT-rich oils for producing edible oils and MCT-rich products. Virgin Coconut Oil (VCO) has the highest MCT levels, followed by Coconut Oil (CNO) and Refined Palm Kernel Oil (RPKO), with slight variations in monounsaturated fat. Coconut Testa Oil (CTO) has a diverse profile with substantial saturated fats and some monounsaturated and polyunsaturated fats. To enrich MCTs in vegetable oils, we developed an enzymatic process using CNO and RPKO. This process produced TAG

(80%) enriched with approximately 50% of C8:0 and C10:0 fatty acids using Enzyme-1. Enzymatic interesterification and blending were performed with commercial immobilized enzymes. CNO exhibited the highest oxidative stability, followed by Palm Oil (PO), Rice Bran Oil (RBO), Sunflower Oil (SFO), and Fish Oil (FO). We improved methods for detecting, identifying, and quantifying medium-chain triglycerides in edible oils. Blends of "CNO: MCT" had the highest MCFA content at 75%, with enzymatic interesterification increasing MCFA levels to 70%. Blended oils showed higher MCFA levels than interesterified oils. The free fatty acid (FFA) content was slightly higher in interesterified oils compared to native and blended oils, but all were less than 1%. Physicochemical characterization was conducted following FSSAI standards for initial, blended, and interesterified oils, including MCT, CNO, RBO, BC, IC, BR, and IR oils. MCT oil exhibited lower free fatty acid content, while interesterified oils showed higher free fatty acids compared to initial and blended oils. Peroxide values were within the limits specified by FSSAI. The iodine value for RBO was high (97.46 cg I2/g) due to the presence of unsaturated fatty acids, whereas MCT showed a lower iodine value (0.11 cg I2/g) owing to the presence of saturated fatty acids. Phytonutrient content analysis revealed that MCT oil contained minimal phytonutrients after blending and interesterification. MCT-enriched oils exhibited nutraceutical potential and health benefits. TAG molecular species were identified using HPLC and HRMS. MCT oil showed only four major peaks: Tricaprylin (C8:0), Tricaprin (C10:0), Dicaprylin monocaprin (C8:0-10:0-8:0), and Dicaprin monocaprylic (C10:0-8:0-10:0), with Dicaprylin monocaprin being the predominant TAG consisting of ECN-26 (45.87%). Blending and interesterification of MCT-based enriched oils maintained these four major peaks. During interesterification, TAG species of long-chain fatty acids were elongated and slightly increased compared to blended oils. TLC profiles showed the formation of DAG, which was consistent with observations from HRMS and HPLC analysis.

We successfully developed a vegan MCT-rich low-fat mayonnaise by combining structured lipids with prebiotic dietary components and nutraceuticals. MCTs provided the desired properties for mayonnaise. We prepared four types of vegan mayonnaise: 100% soybean oil (control), 100% sunflower oil (SFO), a blend of coconut oil and sunflower oil, and a blend of rice bran oil and sunflower oil. Sensory evaluation indicated that the mayonnaise containing MCFA was well-received. MCTs proved to be effective substitutes, providing the desired physicochemical properties for the mayonnaise. The proposed low-fat prebiotic mayonnaise lacks synthetic chemicals or harsh chemical processing. We assert that dietary management for maintaining a healthy body weight can help reduce serum circulatory free fatty acid

content. The physicochemical characterization of the mayonnaise confirmed that all parameters met FSSAI guidelines.The developed product offers reduced fat, improved nutrition, and prebiotics, along with weight management benefits. We conducted an in vivo preclinical study using an animal model to assess the product's toxicity and efficacy to validate our hypothesis.

In vivo evaluation of the product was conducted in both normal and diet-induced obese C57BL/6J (B6) mice. The mayonnaise variants included sunflower oil-based, coconut oil-based, rice bran oil-based, and commercial mayonnaise (low fat and high fat). The modified vegetable oil, rich in medium-chain triglycerides, exhibited a positive effect on the mice, notably leading to significantly lower weight gain compared to other mayonnaise types. The circulatory lipid levels, including triglycerides, cholesterol, LDL, and HDL, were significantly lower with the "CFTRI formulation" compared to commercial and sunflower oil-based mayonnaise. The supplementation of the product to normal mice demonstrated the maintenance of a healthy body weight, while in diet-induced obese mice, a positive impact on circulatory lipids was observed.

CONCLUSION

The present study highlights the potential of MCTs to mitigate the adverse health effects of urban living and unhealthy dietary habits. We have developed an enzymatic process for MCT production with C8:0 and C10:0 fatty acids. Our novel approach of blending traditional edible oils with MCTs enhances the health benefits and improves the lipid profile, offering various health advantages. Additionally, we have established an improved method for detecting, identifying, and quantifying medium-chain triglycerides in edible oils. Furthermore, we formulated a vegan, MCT-rich oil-based low-fat mayonnaise, combining easily digestible structured lipids with prebiotic dietary components and nutraceuticals. The present study shows significant benefits in weight management and cardiovascular health in animal models by developing a commercially viable enzymatic process to produce MCT-rich oils for products like low-fat vegan mayonnaise. The envisioned commercial outcome involves formulating a low-fat prebiotic mayonnaise enriched with glycerides of medium-chain fatty acids and a lipase inhibitor, offering a promising solution for maintaining optimal circulatory lipid levels and promoting overall health. The findings suggest that integrating MCT-rich oil-based products into dietary routines can offer substantial health benefits. However, further research is needed to validate these results in human subjects and explore their broader applications in functional food formulations.

Future studies

Currently, we are in the process of securing intellectual property protection, and patents for both the technology and the product will be filed shortly. The future perspective of the study involves the large-scale production of medium-chain triglyceride rice oil and its products, accompanied by process optimization. Human clinical trials will be conducted in collaboration with medical colleges, followed by the commercialization phase. Ultimately, the study aims to facilitate the commercialization of the technology and the product through technology transfer to industries.

List of publications

Moorthy Karthika Selvi, Aishwarya N Nagalapur, P.Vijayaraj. "Comparative Assessment of Edible Oil Oxidative Stability through Accelerated Stability Study." Biomedicine. 2023; 43(6).

Moorthy Karthika Selvi, Aishwarya N Nagalapur, P.Vijayaraj **"**Comparative Study to Assess the Medium-Chain Triglyceride Content in Various Edible Oils for Functional Food Formulation" Medicinal Plants, Vol. 15 (4), 2023, 619-628.

Moorthy Karthika Selvi & Vijayaraj P. "An improved method for the detection, identification and quantification of medium-chain triglycerides in edible oils". Food frontiers (Communicated).

Moorthy Karthika Selvi & Vijayaraj P "Enriching medium-chain triglycerides in the edible oils by an enzymatic process". Food chemistry (Communicated).

Conference/ Workshop / Poster

1. Moorthy Karthika Selvi, Navya N, Anjali P and P Vijayaraj. Nutraceutical intervention for the management of obesity and insulin resistance by lipase inhibition. 7thBioprocessing India Conference. CSIR-CFTRI, Mysore. 14th -16th Dec. (BBT-029), 120.
2. Moorthy Karthika Selvi and P.Vijayaraj. "Lipidome profiling and oxidative stability of oils enriched with medium chain triglycerides".29th ICFOST organized by AFST (I)-HQ (Mysuru), Kollam & Cochin chapter during 5th -7th January 2023.
3. Moorthy Karthika Selvi and P.Vijayaraj ."An improved method for the detection, identification, and quantification of medium-chain triglycerides in edible oils". 9th International Food Convention. CSIR-CFTRI Campus, Mysore.7th -10th December 2023.

Reference

- Akoh C. C, Min D.B (2002) Food Lipids: Chemistry, Nutrition and Biotechnology. Marcel Dekker Inc. Madison Avenue, NY, USA. ISBN: 0-8247-0749-4. pp 54.
- Akoh. C. C., & Min. D.B (2008). Food lipids: Chemistry, Nutrition and Biotechnology, 3rd edition CRC press, Taylor and Francis group. O'Keffee, Nomenclature and classification of lipids. 3-35.
- Ashton, J. S., Roberts, J. W., Wakefield, C. J., Page, R. M., MacLaren, D. P., Marwood, S., & Malone, J. J. (2021). The effects of medium chain triglyceride (MCT) supplementation using a C8: C10 ratio of 30: 70 on cognitive performance in healthy young adults. Physiology & Behavior, 229, 113252.
- Babayan, V. K. (1987). Medium chain triglycerides and structured lipids. *Lipids*, *22*(6), 417-420.
- Bach, A. C., & Babayan, K. Medium-chain triglycerides: an update. American Journal of Clinical Nutrition, 1982, 36,950–962.
- Boemeke, L., Marcadenti, A., Busnello, F. M., & Gottschall, C. B. A. (2015). Effects of coconut oil on human health. *Open Journal of Endocrine and Metabolic Diseases*, *5*(07), 84.
- Denke, M. A., & Grundy, S. M. (1992). Comparison of effects of lauric acid and palmitic acid on plasma lipids and lipoproteins. The American journal of clinical nutrition, 56(5), 895-898.
- DiNicolantonio, J. J., & Lucan, S. C. (2014). The wrong white crystals: not salt but sugar as aetiological in hypertension and cardiometabolic disease. Open heart, 1(1), e000167.
- Dorni, C., Sharma, P., Saikia, G., & Longvah, T. (2018). Fatty acid profile of edible oils and fats consumed in India. Food chemistry, 238, 9-15.
- Dorni, C., Sharma, P., Saikia, G., &Longvah, T. (2018). Fatty acid profile of edible oils and fats consumed in India. Food chemistry, 238, 9-15.
- Duttaroy AK (2021) Bioactive lipids in metabolic syndromes and hemostatic factors and fibrinolysis. In: Duttaroy AK (ed) Evidence-based nutrition and clinical evidence of bioactive foods in human health and disease. Academic Press, London, pp 63–78
- Esperon-Rojas AA, Mendoza-Sanchez LG, Garcia HS (2022) In: Kuddus M, Aguilar CN (eds) Value-addition in food

- products and processing through enzyme technology.
- Academic Press, pp 221–233
- Flickinger, B. D., & Huth, P. J. (2004). Dietary fats and oils: technologies for improving cardiovascular health. Current atherosclerosis reports, 6(6), 468-476.
- Haslam, D. (2016). Weight management in obesity–past and present. International journal of clinical practice, 70(3), 206-217.
- Jadhav, H., &Annapure, U. (2021a). Greener route for intensified synthesis of Tricaprylin using Amberlyst-15. Journal of Chemical Sciences, 133, 1-7.
- Jadhav, H., Gogate, P., &Annapure, U. (2021b). Intensification of synthesis of triglyceride of Decanoic acid in the presence of amberlyst 15 as catalyst based on the use of ultrasound and microwave irradiations. Chemical Engineering and Processing-Process Intensification, 165, 108424.
- Jensen, R. G. (2002). The composition of bovine milk lipids: January 1995 to December 2000. Journal of dairy science, 85(2), 295-350.
- Jin, X., Qiu, T., Li, L., Yu, R., Chen, X., Li, C., ... & Jiang, T. (2023). Pathophysiology of obesity and its associated diseases. Acta Pharmaceutica Sinica B, 13(6), 2403-2424.
- Kinsella, J. E. (1988). Food lipids and fatty acids. Food Technol., 42, 124-145.
- Krishna, A. G., Gaurav, R., Singh, B. A., Kumar, P. P., & Preeti, C. (2010). Coconut oil: chemistry, production and its applications-a review. Indian Coconut Journal, 53(3), 15-27.
- Lee, T. W. (1986). Quantitative determination of medium chain triglycerides in infant formula by reverse phase HPLC. Journal of the American Oil Chemists' Society, 63(3), 317-320.
- Liu, Y., Lu, X., Li, X., Du, P., & Qin, G. (2020). High-fat diet triggers obesity-related early infiltration of macrophages into adipose tissue and transient reduction of blood monocyte count. Molecular immunology, 117, 139-146.
- Li, X., Liu, Q., Pan, Y., Chen, S., Zhao, Y., & Hu, Y. (2023). New insights into the role of dietary triglyceride absorption in obesity and metabolic diseases. Frontiers in Pharmacology, 14, 1097835.
- Marten, B., Pfeuffer, M., &Schrezenmeir, J. (2006). Medium-chain triglycerides. International Dairy Journal, 16(11), 1374-1382.
- Mett, J., & Muller, U. (2021). The medium-chain fatty acid decanoic acid reduces oxidative stress levels in neuroblastoma cells. Scientific Reports, 11(1), 6135.

- Pandya AJ, Haenlein GFW (2009) In: Park YW (ed) Bioactive components in milk and dairy products. Wiley, pp 105–158
- Petrauskaite V., De Greyt, W. F., &Kellens, M. J. Physical refining of Coconut oil: effect of crude oil quality and deodorization conditions on neutral oil loss. Journal of the American Oil Chemists' Society, 2000, 77(6), 581-586.
- Rustan, A. C., & Drevon, C. A. (2001). Fatty acids: structures and properties. e LS.
- St-Onge, M. P., & Jones, P. J. (2002). Physiological effects of medium-chain triglycerides: potential agents in the prevention of obesity. The Journal of nutrition, 132(3), 329-332.
- Traul, K. A., Driedger, A., Ingle, D. L., &Nakhasi, D. Review of the toxicologic properties of medium-chain triglycerides. Food and chemical toxicology, 2000, 38(1), 79-98.
- Turpeinen, A., &Merimaa, P. (2011). Functional fats and spreads. In Functional foods (pp. 383-400). Woodhead Publishing.
- Wallace, T. C. (2019). Health effects of coconut oil-A narrative review of current evidence. Journal of the american college of nutrition, 38(2), 97-10.
- Willett, W. C. (1998). Is dietary fat a major determinant of body fat? The American journal of clinical nutrition, 67(3), 556S-562S.
- Yuan, T., Geng, Z., Dai, X., Zhang, X., Wei, W., Wang, X., & Jin, Q. (2020). Triacylglycerol containing medium-chain fatty acids: Comparison of human milk and infant formulas on lipolysis during in vitro digestion. Journal of agricultural and food chemistry, 68(14), 4187-4195.
- Zambiazi, R. C., Przybylski, R., Zambiazi, M. W., & Mendonca, C. B. (2007). Fatty acid composition of vegetable oils and fats. Boletim do Centro de Pesquisa de Processamento de Alimentos, 25(1).
- Zentek, J., Buchheit-Renko, S., Ferrara, F., Vahjen, W., Van Kessel, A. G., & Pieper, R. (2011). Nutritional and physiological role of medium-chain triglycerides and medium-chain fatty acids in piglets. Animal Health Research Reviews, 12(1), 83-93.

Signature of student
(**Moorthy Karthika Selvi**)

Signature of Guide
(**Dr. P. Vijayaraj**)